EFFECTS OF DIETARY NUCLEOTIDES ON GROWTH, IMMUNOLOGY, AND DISEASE RESISTANCE OF JUVENILE NILE TILAPIA (*Oreochromis niloticus)*

A Thesis

by

MARITZA ANGUIANO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2011

Major Subject: Wildlife and Fisheries Sciences

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Co-Chairs of Committee,	Delbert M. Gatlin, III
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ABSTRACT

Effects of Dietary Nucleotides on Growth, Immunology, and Disease Resistance of Juvenile Nile Tilapia (*Oreochromis niloticus)*. (December 2011)

Maritza Anguiano, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Delbert Gatlin
Dr. Alejandro Buentello

In order to improve production efficiency and profitability in tilapia aquaculture, further research is needed to develop methods to improve weight gain, feed utilization, and immune function of these fish. In this regard, numerous studies with several fish species have reported that dietary nucleotides can enhance growth performance, immune responses and disease resistance. Therefore, two feeding experiments were conducted to investigate the effects of a purified nucleotide mix on juvenile Nile tilapia, *Oreochromis niloticus*.

A basal diet was formulated to contain 34% crude protein from fishmeal and soybean meal. A nucleotide mix containing salts of cytidine, uridine, adenosine, inosine, guanosine, and thymine was supplemented to the basal diet at 0.5, 1 and 2% of dry weight. In the first experiment, three replicate groups of 15 fish were fed the experimental diets. At the end of 8 weeks, weight gain, feed efficiency and survival were computed. Blood samples were analyzed for neutrophil oxidative radical production and plasma lysozyme activity. In the second trial, three replicate groups of 20 fish were fed the same experimental diets. At the end of 4 weeks, blood and kidney samples were

analyzed for macrophage extracellular and intracellular superoxide anion production, blood neutrophil oxidative radical production, plasma lysozyme activity, and peripheral blood lymphocyte proliferation. Then, 12 fish per treatment were challenged with *Streptococcus iniae*, via intraperitoneal injection, and mortality was recorded for 21 days.

Results showed that none of the nucleotide-supplemented diets induced significant ($P < 0.05$) effects on growth performance. On the other hand, the 0.5% treatment produced significantly ($P < 0.05$) higher intracellular superoxide anion (O_2^-) production and both the 0.5 and 1% treatments significantly ($P < 0.05$) increased lymphocyte proliferation. The disease challenge failed to show significant survival differences among treatments; however, the 2% nucleotide treatment tended to produce higher survivability. Results from both experiments lead to the conclusion that this particular nucleotide mix does not provide marked improvements in growth performance and disease resistance; however, dietary nucleotide supplementation did affect some components of the immune system of Nile tilapia.

DEDICATION

To my caring husband, Angel, to my parents, and to all my family.

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you for being there and for your support. Finally, I would like to thank my husband, Angel. Thank you for being there so many countless times and in so many ways.

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1. INTRODUCTION

Tilapia is the common name applied to fish species in the genera *Oreochromis, Sarotherodon* and *Tilapia* (Watanabe et al., 2002). Today, tilapia have become one of the most important food fish in the world with total sales exceeding $2 billion per year (FAO, 2011; Fitzsimmons, 2000, 2006; Watanabe et al., 2002). Tilapia aquaculture, in particular of *Oreochromis* sp., is widely distributed throughout the world because of the ability of the species to adapt to many different culture conditions (Watanabe et al., 2002). However, as culture practices intensify, fish often become more susceptible to disease as a result of crowding stress, poor water quality, and handling strain (Shelton and Popma, 2006). While tilapia are more resistant than other fish species to viral, bacterial and parasitic diseases, infectious diseases have increased as a result of increased culture densities (Shelton and Popma, 2006; Watanabe et al., 2002). Bacterial infections from species in the genera *Streptococcus* have caused heavy losses in intensively cultured tilapia but disease caused by species of the genera *Edwardsiella*, *Aeromonas*, *Pseudomonas*, and *Vibrio* has also increased (Shelton and Popma, 2006; Watanabe et al., 2002). Disease can become especially problematic for farms in the U.S. as, currently, there are no Food and Drug Administration (FDA)-approved antibiotics to treat bacterial infections of tilapia (Li et al., 2006).

This thesis follows the style of the journal Aquaculture.

The tilapia industry is still growing and is expected to continue to experience rapid growth with production becoming more intensive (Fitzsimmons, 2000). Further research is needed to improve production efficiency and profitability through developing methods by which fish have faster growth, improved feed utilization, and an enhanced immune function (Watanabe et al., 2002).

Recent research on the use of nucleotides as diet supplements suggests that these compounds may enhance fish growth and immunity such that improved production may be possible in future aquaculture operations, including those involving tilapia.

Nucleotides are compounds of major importance to cellular function and metabolism (Cheng et al., 2011). They are composed of a nitrogenous base, a pentose sugar and one or more phosphate groups with the nitrogenous base being a purine or a pyrimidine (Lehninger et al., 2005). These compounds are also the building blocks of nucleic acids, both DNA and RNA, and also provide metabolic energy. Additionally, nucleotides may serve as coenzyme components, regulatory molecules, and participate in protein synthesis and immunocompetence (Carver and Walker, 1995; Cheng et al., 2011; Mateo, 2005).

Nucleotides were thought to be non-essential nutrients because the synthesis of purines and pyrimidines through de novo or salvage pathways are typically sufficient (Carver and Walker, 1995; Li and Gatlin, 2006). However, during times of rapid growth, stress, or in immuno-compromised animals, normal synthesis of nucleotides may not be sufficient and dietary supplementation has proven to be beneficial (Carver and Walker, 1995; Mateo, 2005; Rudolph, 1994). Several studies with humans and animals have

shown that dietary supplementation can improve infant development, immune function, intestinal health as well as growth (Grimble and Westwood, 2000; Holen et al., 2006; Mateo, 2005; Pickering et al., 1998; Pirgozliev et al., 2009).

Fish in culture systems typically experience periods of rapid growth and can be exposed to stressful conditions (Shelton and Popma, 2006). While initial studies on dietary nucleotide supplementation in fish were focused on the possible improvement in palatability of diets (Li and Gatlin, 2006; Mackie and Adron, 1978), recent findings have shown that dietary nucleotides can produce similar improvements in fish as those found in humans and terrestrial animals. Research with several fish species including hybrid tilapia (*Oreochromis niloticus* x *O. aureus*), red drum (*Sciaenops ocellatus*), and Atlantic salmon (*Salmo salar)* have found that dietary nucleotide supplementation can influence growth, immune response and intestinal morphology (Burrells et al., 2001a; Cheng et al., 2011; Li et al., 2007; Ramadan et al., 1994).

There also have been a limited number of studies in which the supplementation of nucleotides has been evaluated with tilapia (Ramadan et al., 1991; 1994). However, these studies used commercial nucleotide products for which limited information was provided. This makes it difficult to compare results and to fully understand the potential mechanisms that might be affecting the fish. Several other studies have obtained results with specific nucleotide mixtures for red drum (Li et al., 2007) and grouper, *Epinephelus malabaricus*, (Li et al., 2009), but the same information is lacking for tilapia. Therefore, the present study was conducted to provide information pertaining to a purified

nucleotide mixture and their effects on tilapia physiology so that results may be applied efficiently in the tilapia industry and also direct future research.

1.1 Objective

Based on the previous information, the objective of this study was to evaluate the effects of three concentrations (0.5, 1, and 2% of diet) of a purified dietary nucleotide mixture on juvenile Nile tilapia with respect to growth, non-specific immunity and disease resistance.

2. MATERIALS AND METHODS

2.1 Feeding trials

Two feeding trials were conducted to evaluate the effects of three nucleotide concentrations (0.5, 1, and 2% of diet) from a purified mixture, on juvenile Nile tilapia. All diets were formulated to have 34% crude protein utilizing menhaden fishmeal and de-hulled, solvent-extracted soybean meal as protein sources, 8.2% lipid, and 2.9 kcal of estimated digestible energy g^{-1}. The dietary nucleotide mixture was made using five nucleotide 5'-monophosphate salts (all from Sigma-Aldrich, St. Louis, MO, USA) of cytidine, uridine, adenosine, inosine, guanosine and one nucleo-base, thymine. These nucleotides were mixed in equal amounts at 10% of the weight of the final purified mixture. The final purified mixture was made by triple binding the individual nucleotide salts with casein, gelatin, and carboxymethyl cellulose (CMC, U.S. Biochemical Corp., Cleveland, OH, USA), freeze drying the mixture, and finally grounding it into a fine powder. The diets were maintained isonitrogenous and isocaloric by adjusting the amounts of casein, gelatin and CMC in all diets (Table 1). The test diets were supplemented with the nucleotide mixture at 0.5, 1 and 2% of dry weight with final free nucleotide concentrations estimated at 0.5, 1 and 2 g/kg. The basal diet contained no nucleotide mixture and served as the control.

Both trials were conducted at the Texas A&M University Aquacultural Research and Teaching Facility in aquaria maintained indoors in a climate-controlled laboratory. The first trial was conducted in 38-L aquaria to monitor growth performance of fish fed

the experimental diets over an 8-week period. The second trial was conducted in 110-L aquaria over a 4-week period with a different stock of tilapia that was subjected to a controlled bacterial challenge.

Table 1. Nucleotide test diet formulations.

Ingredient	Basal	0.5%	1%	2%
Menhaden fishmeal	11.0	11.0	11.0	11.0
Soybean meal (de-hulled)	45.4	45.5	45.4	45.5
Dextrin	22.0	22.0	22.0	22.0
Vitamin premix	3.0	3.0	3.0	3.0
Mineral premix	4.0	4.0	4.0	4.0
CMC	3.0	3.0	3.0	3.0
Calcium phosphate	1.0	1.0	1.0	1.0
Soy oil	5.9	5.9	5.9	5.9
DL-Methionine	0.2	0.2	0.2	0.2
Celufil	2.5	2.4	2.5	2.4
Casein/Gelatin/CMC mix	2.1	1.5	1.0	0.0
Nucleotide mix	0.0	0.5	1.0	2.0
Σ	100.0	100.0	100.0	100.0

Water flow into the aquaria for both trials was maintained at approximately 1 L min^{-1} via a recirculating system to maintain adequate water quality through biological and mechanical filtration. Salinity was maintained near 5 g L^{-1} using well water and synthetic sea salts. Dissolved oxygen levels were maintained near air saturation using low-pressure electrical blowers and air stones. Water temperature was kept by controlling ambient temperatures with dual air conditioning units at 25 ± 2 °C throughout both trials. A 12-hour light and 12-hour dark photoperiod was maintained with fluorescent lights controlled by automatic timers.

For the first trial, 180 all-male, juvenile tilapia (*Oreochromis niloticus,* 3-4 g and 2 weeks old) were obtained from Til-Tech Aquafarms, Robert, LA. An all-male population was used as they provide relatively uniform growth and a narrow distribution in size at harvest as opposed to a wide distribution typically observed with mixed-sex tilapia (Shepard et al., 2006). Fish of homogeneous size (average weight 3.4 g per fish) were selected and stocked at 15 fish per 38-L aquaria to assure a level of variation less than 5% among cumulative fish weights. The tilapia were conditioned for 1 week and fed to satiation with the basal diet. At the end of the conditioning period, three replicate aquaria of fish were randomly assigned to each dietary treatment. The fish were fed at the same fixed rate (initially 6% of body weight), twice daily (morning and evening, 7 days a week) and this ration was adjusted weekly to achieve apparent satiation with no feed waste. Growth and feed efficiency were monitored by collectively weighing the fish from each aquarium every week. Mortality also was monitored daily. The feeding trial continued for 8 weeks.

For the second trial, 240 mixed-sex juvenile tilapia (*Oreochromis niloticus,* 60-70 g and at least 2 months old) were obtained from Simaron Freshwater Fish, Inc., Hempstead, TX as previous research in our laboratory established that this stock of fish was susceptible to *Streptococcus iniae* infections. These fish were of larger size than those for the first trial because previous studies in our laboratory also have indicated that larger fish are needed to provide sufficient numbers of phagocytes for extracellular and intracellular superoxide anion and lymphocyte proliferation assays along with the other analyses. An all-male population was not necessary as weight gain was not recorded for

these fish. Groups of 20 fish were graded by size (average weight 64.5 g) and stocked into the 110-L aquaria. These fish also were subjected to a 1-week conditioning period and fed the basal diet. After the conditioning period, three replicate tanks of fish were randomly assigned to each dietary treatment. These fish were fed to apparent satiation twice daily for 4 weeks after which they were subjected to a controlled disease challenge.

2.2 Disease challenge

At the end of the 4-week feeding period, 12 fish from each aquarium were randomly selected and subjected to a standardized dose of *S. iniae*. First a bacterial isolate from our stock cultures was propagated in sterile, nutritive media (brain-heart infusion [BHI], Becton, Dickinson and Company, Sparks, MD) and passed though fish to assure virulence. A preliminary LD_{50} assay was conducted to evaluate the proper concentration of colony forming units (CFU) needed to cause acute mortality in 50% of the population. Based on this assessment, a concentration of approximately 7.44×10^{11} CFU/mL was made and later confirmed via replicate plate counts. Each fish was intraperitoneally injected (~0.6 mL/10 g of body weight) with BHI broth inoculated with the virulent bacteria. After injection, fish were returned to their respective aquaria, keeping three replicate aquaria of challenged fish per dietary treatment. Twelve fish from the same stock were injected with sterile phosphate buffered saline (PBS, Sigma-Aldrich, St. Louis, MO, USA) and transferred to an aquarium in the same system (Balfry et al., 1997; Martins et al., 2009). These fish served as controls to assure that mortalities in the system were from the disease and not from stress resulting from the

injection or environmental conditions. Mortalities were monitored for 21 days following the injection.

2.3 Sampling

At the end of the conditioning period for the first trial, 10 fish were randomly selected and euthanized using tricaine methanesulfonate (MS-222, 200 mg/L, Western Chemical, Ferndale, WA). These fish served as an initial sample for measuring whole-body proximate composition including dry matter, ash, moisture, lipid and protein using established procedures (Folch et al., 1957; Nematipour et al., 1992).

At the end of the 8-week feeding trial, three fish from each tank (9 fish per treatment) were randomly selected and bled using heparinized needles. Whole-blood was analyzed for neutrophil oxidative radical production and plasma lysozyme activity as described in the next section. After bleeding, fish were euthanized with the same MS-222 overdose and whole-body samples also were analyzed for proximate composition. Each sample was analyzed in duplicate.

At the end of the second feeding trial of 4-weeks duration, three fish were randomly selected from each aquarium. Whole-blood was analyzed as in the previous trial. After bleeding, these fish were euthanized with MS-222 as previously described and the head and trunk kidneys were dissected for isolation of macrophages, after which extracellular and intracellular superoxide anion production was determined. Two more fish were randomly selected from the remaining fish and blood was collected to analyze lymphocyte proliferation, which was determined by measuring bromodeoxyuridine (BrdU) incorporation into proliferating cells as described in the next section.

2.4 Immunological assay procedures

All reagents for immunological procedures were obtained from Sigma-Aldrich, St. Louis, MO, USA unless specified otherwise. Neutrophil oxidative radical production provides a measure of neutrophil activation such that it can be related to the fish's inflammatory reaction (Rumsey et al., 1994). For this test, the blood was analyzed within 10 h according to the procedures of Siwicki et al. (1994). Briefly, 50 µl of blood was mixed and incubated with 50 µl of 0.2% nitro blue tetrazolium (NBT) in PBS for at least 30 min. From this mixture, 50 µl was added to 1 mL of *N,N*- dimethyl formamide and then centrifuged at 3000 x *g* for 5 min. The supernatant was read with a spectrophotometer at 545 nm. The absorbance was converted to NBT units based on a standard curve of NBT diformazan mL^{-1} of blood.

Lysozyme activity provides a measure of the lytic activity against gram-positive and gram-negative bacteria as part of the innate immunity in fish (Saurabh and Sahoo, 2008). For this method, whole-blood was first centrifuged (6000 x *g*, 5 min) to separate the plasma. The plasma was then analyzed for lysozyme activity based on a turbidimetric method as described by Jorgensen et al. (1993). A 25-µl sample of plasma was mixed with a suspension of *Micrococcus lysodeikticus* in PBS. Absorbance (540 nm) was read at 30 s and 5 min. The change in absorbance over time was converted to units per mL.

Extracellular and intracellular superoxide anion (O_2^-) production was evaluated using the procedures of Secombes (1990) as modified by Sealey and Gatlin (2002). This test analyzes the phagocytic activity of kidney macrophages, which are key components

in cellular non-specific immune response (Sealey and Gatlin, 2002). Kidney samples from three fish in each of three replicate aquaria were pooled together for each dietary treatment. Then, the kidneys were homogenized in L-15 media with 2% fetal calf serum (FCS). Macrophages were isolated using a Percoll gradient, re-suspended in L-15 media with 1% FCS and delivered at 2 x 10^6 macrophages per well. These cells were allowed to attach for 2 h, washed, and then supplemented with L-15 media containing 5% FCS.

Extracellular O_2^- was measured as the reduction of ferricytochrome c using phorbol myristate acetate (PMA) to stimulate the cells. Some wells also received superoxide dismutase (SOD) to inhibit the reaction and verify specificity. The wells were read with a multi-scan spectrophotometer at 550 nm every 15 min for 1 h and the results were presented in nmol O_2^-. Intracellular O_2^- was measured by the addition of NBT and PMA. Some wells also received SOD and were used as blanks. The cells were incubated for 45 min and then fixed with 100% methanol. The wells were then washed with 70% methanol and the formazan was solubilized with 2M potassium hydroxide and dimethyl sulfoxide. The wells were read at 620 nm. The results were presented as optical density.

The lymphocyte proliferation assay was based on methods by Gogal et al. (1999), Miller and Clem (1988) and Solis et al. (2007). This assay provides a measure of activation of lymphocytes when stimulated by antigen, which can be related to the immunological competence of the lymphocytes (Janossy and Greaves, 1971). Briefly, sampled blood was pooled by each treatment. Lymphocytes were isolated from whole blood using Lymphoprep™ (Accurate Chemical and Scientific Corp., Westbury, NY,

USA) as the isolation media and suspended in Roswell Park Memorial Institute media (RPMI 1640; Lonza, Walkersville, MD, USA) with 5% fetal bovine serum. Then, 5 x 10^5 lymphocytes were delivered per well into round-bottom culture plates in the presence or absence of mitogen. Concanavalin A (Con A; MP Biomedical, Solon, OH, USA), which is known to stimulate T lymphocyte proliferation (Agbede et al., 2005; Miller and Clem, 1988), was tested at 25, 50, and 75 μg/mL. Each test concentration of Con A was analyzed with three replicate wells. Cultures were incubated for 2 days at 28°C in a humidified 5% CO_2-95% air atmosphere. A BrdU cell proliferation assay kit (Millipore, Bellerica, MA, USA) was used to determine proliferation. BrdU was added to the suspension to be incorporated into proliferating cells for 24 h. After the incubation period, BrdU incorporation was followed according to the manufacturer's instructions by measuring optical density at 450 nm.

2.5 Statistical analyses

Analysis of variance (ANOVA) was used to determine significant ($P < 0.05$) dietary effects with regard to the performance indices, immunological assays and disease resistance. All statistical analyses were performed using SAS software (version 9.2, SAS Institute, Inc., Cary, NC, USA).

3. RESULTS

3.1 Fish growth performance and proximate composition

During the course of the 8-week feeding period in trial 1, the weight gains were significantly different from each other at weeks 2 through 7 (Figure 1). The average weight gain for the fish fed the basal diet tended to be higher than the nucleotide-supplemented diets and was significantly higher from 6 to 7 weeks. The average weight gain for the fish fed the 0.5% nucleotide supplementation diet tended to be the least of all dietary treatments and was significantly lower from 2 to 6 weeks. However, at the end of 8 weeks, fish fed all of the diets had similar weight gain values that were not statistically different.

At the end of the 8-week feeding experiment feed efficiency and survival values also were calculated for each dietary treatment (Table 2). Conspicuously, although fish fed the basal and the 1% nucleotide supplemented diets had the greatest numerical increase in weight gain, both also had the least survival at 87% and 84%, respectively. Fish fed the diet supplemented with 0.5% nucleotides had the highest feed efficiency; however, there were no significant differences among fish fed the various dietary treatments in weight gain as well as feed efficiency and survival. Finally, none of the dietary treatments resulted in any significant changes to the whole-body composition of the fish (Table 3). Dry matter, ash, lipid and crude protein of carcass tissues were similar among all dietary treatments.

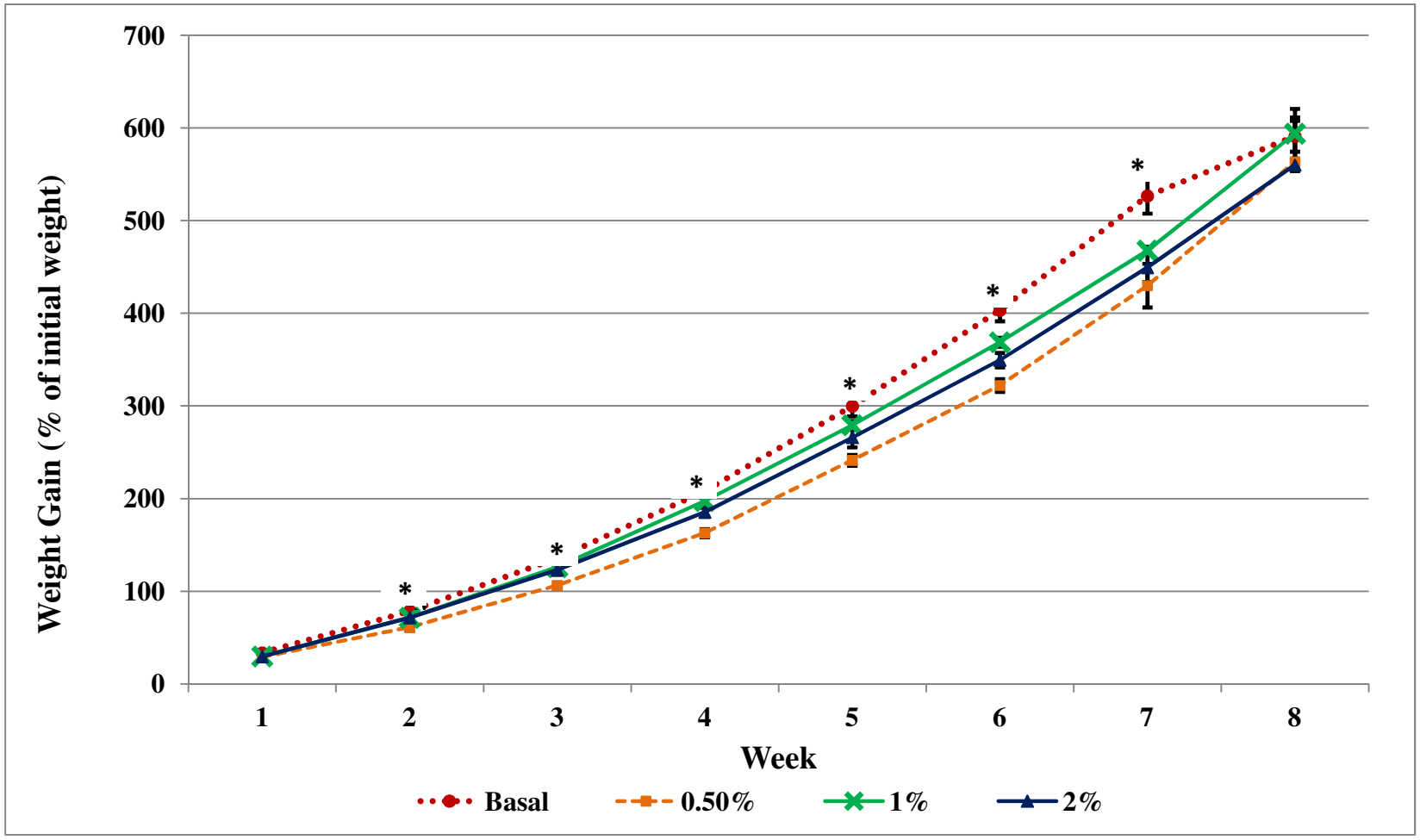

Figure 1. Average weight gain of fish fed each dietary treatment over 8 weeks. Each average is from three replicate tanks. Mean ± SE. *Weight gain significantly different ($P<0.05$) among dietary treatments based on ANOVA.

Table 2. Weight gain, feed efficiency ratio and survival at the end of 8 weeks.

Diet	Weight gain (% of initial wt)	Feed efficiency (g gain/g dry feed)	Survival (%)
Basal	591	0.72	86.7
0.5%	563	0.82	88.9
1%	594	0.79	84.4
2%	560	0.77	88.9
Pr > F[1]	0.77	0.46	0.82
Pooled SE[2]	14.43	0.02	1.92

[1] Significance probability associated with the F statistic
[2] Pooled standard error

Table 3. Proximate composition of whole-body tissues of tilapia at the end of 8 weeks [1].

Diet	Dry matter (%)	Ash (%)	Lipid (%)	Crude protein (%)
Basal	28.5	3.7	6.1	18.9
0.5%	26.7	3.2	6.7	18.2
1%	28.2	3.9	6.2	18.7
2%	27.3	3.5	7.6	17.6
Pr > F[2]	0.41	0.26	0.42	0.33
Pooled SE[3]	0.39	0.11	0.35	0.26

[1]Average of three pooled fish from each of three replicate aquaria.
[2] Significance probability associated with the F statistic
[3] Pooled standard error

3.2 Immune cell responses

At the end of the 8-week feeding trial, blood was taken from three random fish from each aquarium and analyzed for neutrophil oxidative radical production (NBT) and plasma lysozyme activity (Table 4). None of the dietary treatments produced significant changes in NBT or lysozyme activity. However, a similar trend for weight gain was also found for NBT. Fish fed both the basal diet and the diet supplemented with 1%

nucleotides tended to have the highest NBT response. That trend did not exist with regard to lysozyme activity.

Table 4. Neutrophil oxidative radical production and lysozyme activity after 8 weeks of feeding.

Diet	NBT[1] (mg NBT/mL blood)	Lysozyme (U/mL)
Basal	1.08	49.4
0.5%	0.92	51.1
1%	1.09	38.3
2%	0.90	43.3
Pr > *F*[2]	0.11	0.67
Pooled SE[3]	0.03	4.05

[1] NBT = neutrophil oxidative radical production
[2] Significance probability associated with the F statistic
[3] Pooled standard error

At the end of the 4-week feeding trial, blood and kidney samples were analyzed for NBT, lysozyme activity, macrophage extracellular and intracellular superoxide anion (O_2^-) production and lymphocyte proliferation (Table 5). None of the dietary treatment resulted in significant ($P > 0.05$) changes to NBT or lysozyme activity as was also seen in the 8-week feeding experiment. However, a similar trend was seen in NBT as in the 8-week feeding trial with fish fed the basal diet and the 1% nucleotide supplemented diet tending to have higher NBT values than those fed the other diets.

While not statistically different, both the 0.5 and 1% nucleotide-supplemented diets tended to produce higher lysozyme activity. This trend also was evident in the results for lymphocyte proliferation. Both 0.5 and 1% nucleotide-supplemented diets produced significantly ($P< 0.05$) higher lymphocyte proliferation values than the basal

diet. Fish fed the 0.5% nucleotide supplemented diet also had significantly ($P< 0.05$) higher intracellular O_2^- production than those fed the basal diet. Finally, extracellular O_2^- production did not show any significant differences among the dietary treatments.

Table 5. Neutrophil oxidative radical production, lysozyme activity, macrophage extracellular and intracellular superoxide anion production, and lymphocyte proliferation after 4 weeks of feeding diets with different levels of nucleotides [1].

Diet	NBT[2] (mg NBT/mL blood)	Lysozyme (U/mL)	Extracellular superoxide anion (nmol O_2^-)	Intracellular superoxide anion (OD[3] at 620 nm)	Lymphocyte proliferation (OD at 450 nm)
Basal	0.63	51.7	0.62	0.16[b]	0.09[b]
0.5%	0.55	64.4	0.70	0.24[a]	0.15[a]
1%	0.68	69.4	0.59	0.21[ab]	0.15[a]
2%	0.51	51.7	0.68	0.20[ab]	0.13[ab]
Pr > F[4]	0.29	0.76	0.24	0.03	0.05
Pooled SE[5]	0.03	7.22	0.02	0.01	0.01

[1] Different superscripts grouped by Duncan's multiple range test ($P < 0.05$).
[2] NBT = neutrophil oxidative radical production
[3] OD = optical density
[4] Significance probability associated with the F statistic
[5] Pooled standard error

3.3 Disease challenge

At the end of the 4-week feeding experiment, 12 fish from each aquarium (total 36 fish per treatment) were intraperitoneally injected with *S. iniae* and mortality was monitored for 21 days. Over this period, mortality slowly increased among all dietary treatments (Figure 2). Fish fed the basal diet and the 2% nucleotide-supplemented diet both had the least cumulative average mortality throughout the observation period, while fish fed both the 0.5 and 1% nucleotide-supplemented diets had the highest mortality. However, due to the high degree of variance among fish in the three replicate aquaria, there were no significant ($P > 0.05$) differences in mortality among dietary treatments at the end of the monitoring period (Table 6).

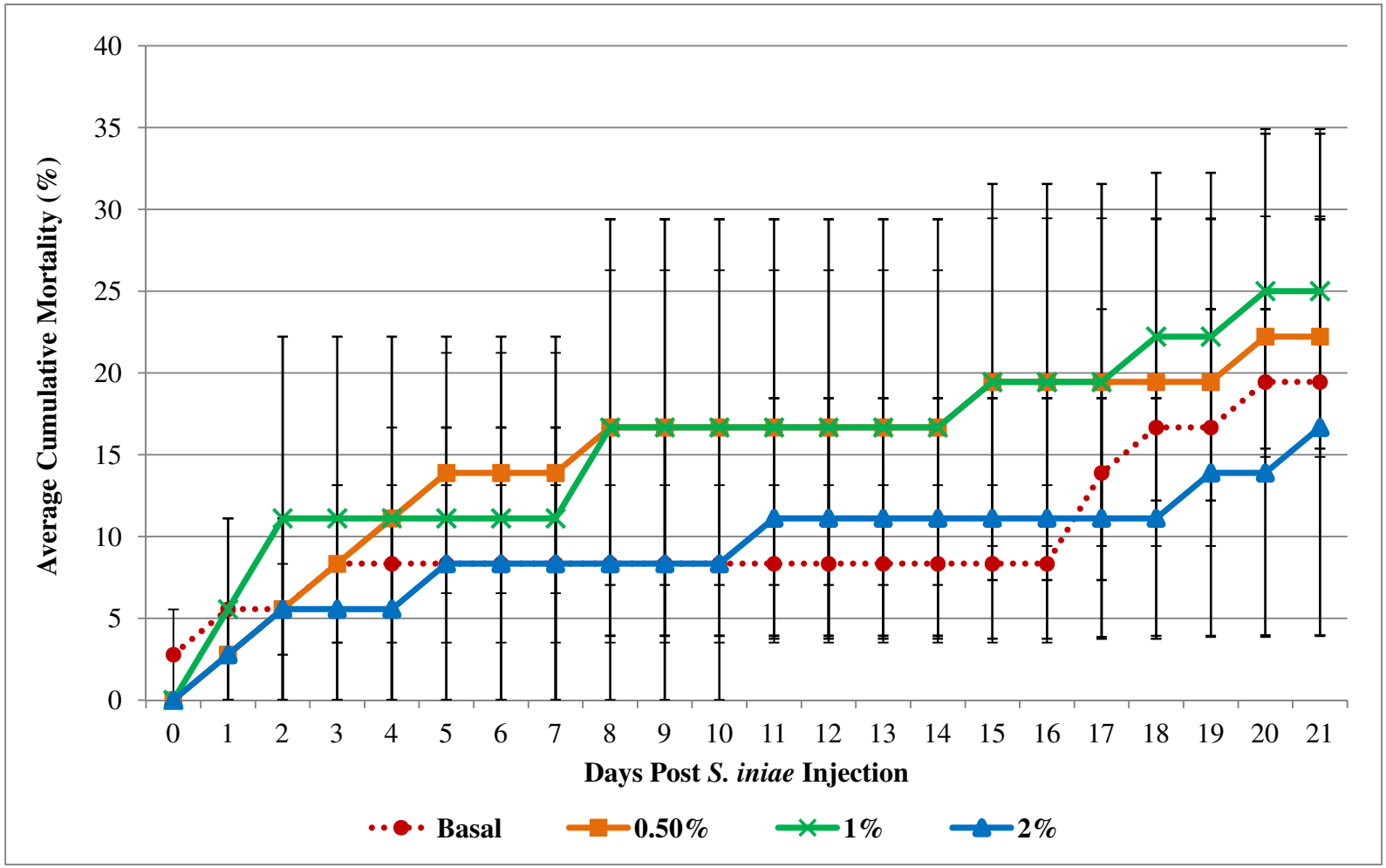

Figure 2. Cumulative mortality of fish fed each dietary treatment after injection of *Streptococcus iniae*. The cumulative mortality is from three replicate tanks. Mean ± SE.

Table 6. Average fish mortality (± S.E.) after challenge with *Streptococcus iniae*[1].

Diet	Mortality (%)
0.5%	22.2±15.5
1%	25.0±7.3
2%	16.7±9.6
Basal	19.4±12.7
Pr>F[2]	0.96
Pooled SE[3]	5.85

[1] Average of three replicate tanks
[2] Significance probability associated with the F statistic
[3] Pooled standard error

4. DISCUSSION

Research on several fish species has indicated that dietary nucleotides may have beneficial effects. Dietary nucleotide supplementation in fish has resulted in improved growth, increased disease resistance, and increased immune response in several species (Li and Gatlin, 2006) . The two feeding trials in the present study were conducted in order to study the effects of a purified nucleotide preparation on growth performance, the immune system and disease resistance of tilapia.

Over the course of 8 weeks, the nucleotide treatments did not influence fish weight gain. Currently, only one study has documented positive effects of dietary nucleotides on growth parameters of tilapia. Ramadan et al. (1991) reported that Ascogen 'S' supplementation resulted in a significant increase in absolute body weight in hybrid tilapia (*Oreochromis niloticus* x *O. aureus*). Others studies in grouper, rainbow trout, and Atlantic salmon (Burrells et al., 2001b; Lin et al., 2009; Tahmasebi-Kohyani et al., 2011) also reported an increase in weight gain after 8 weeks of feeding nucleotide-supplemented diets.

On the other hand, there are several studies that indicate that dietary nucleotide supplementation may not positively enhance weight gain. Studies on red drum reported marginal or no effect on weight gain (Cheng et al., 2011; Li et al., 2007) and similar results were found in hybrid striped bass (*Morone chrysops* x *M. saxatilis*), red-tailed black shark (*Epalzeorhynchos bicolor*), and channel catfish (*Ictalurus punctatus*) (Li et al., 2004; Russo and Yanong, 2006; Welker et al., 2011). Several authors also have

suggested that commercial nucleotide products may have other components which also provide an added effect with the nucleotides (Lin et al., 2009; Welker et al., 2011). However, as positive and negative results in weight gain previously mentioned were found both in studies with commercial products and purified nucleotides, it cannot be assumed that either commercial or purified nucleotide produce greater effects on weight gain.

In contrast to the positive effects previously mentioned, Welker et al. (2011) documented several measured parameters were inversely related to the concentration of a nucleotide supplementation. The nucleotide supplementation in the study of Welker et al. (2011) was similar to the present study in that nucleotides were added as purified monophosphate salts to the basal diet. In that study, channel catfish showed less weight gain as the nucleotide concentration increased. This suggests there may be a maximum concentration from which the fish will benefit before a negative effect is observed.

Feeding nucleotides for 8 weeks also did not produce any significant effects on feed efficiency, survival, and proximate composition of tilapia in the present study. Very few studies have reported significant effects related to feed efficiency (Lin et al., 2009; Tahmasebi-Kohyani et al., 2011). Although not significant, the nucleotide-supplemented diets in the present study did generally support higher feed efficiency. This is consistent with the results found in several studies that also report no significant effect on feed efficiency (Cheng et al., 2011; Li et al., 2004; 2007; 2005; Welker et al., 2011) .

Cheng et al. (2011), Lin et al. (2009), and Welker et al. (2011) also reported a similar general trend in survival, though again not significant. The present study did not

see any trend and instead, fish fed the basal and 1% nucleotide diets had the highest weight gains together with lowest survivals. Finally, Li et al. (2005) found a significant increase in the whole-body lipid content of red drum fed the nucleotide product, Optimûn, compared to that of fish fed a basal diet. However, in another study Li et al. (2007) failed to see any changes in whole-body composition using purified nucleotide treatments in the diet of red drum similar the results of whole-body composition found in the present study.

The present experiment also produced some limited evidence of stimulation to the innate and adaptive immune responses of tilapia. Nucleotide supplementation for both 4 and 8 weeks did not produce significant changes in neutrophil oxidative radical production (NBT). However, in both trials fish fed the basal and 1% nucleotide-supplemented diet had the highest responses, similar to the trend found for weight gain. On the other hand, lysozyme at both 4 and 8 weeks did not show any apparent trends in response to nucleotide supplementation as did extracellular superoxide anion production.

All the nucleotide-supplemented diets tended to result in higher intracellular anion production and lymphocyte proliferation of fish compared to those fed the basal diet. The results also indicated there was an inverse relationship in the nucleotide supplementation level and the immune cell response. Although 0.5 and 1% nucleotide supplementation provided higher responses in intracellular superoxide anion production and lymphocyte proliferation, none conferred disease resistance against *Streptococcus iniae* as fish fed either of these diets experienced the highest mortality over the 21-day post injection period. On the other hand, fish fed the diet with 2% nucleotide

supplementation had the least mortality even though in the immunological analyses, results suggested this diet had the lowest protective response.

Only one study has reported nucleotide effects on the immune system of tilapia. Ramadan et al. (1994) documented that Ascogen fed at 5 g/kg significantly increased mean antibody titers in tilapia after 6 weeks, and also increased survival in *Aeromonas hydrophila*-vaccinated fish. That concentration was much higher than the highest concentration (2 g/kg) in the present study, yet the present study showed a negative trend with higher dosage. Differences between this study and that of Ramadan et al. (1994) may be attributed to the use of a commercial product in the former study, which may have included other additives besides nucleotides that could have contributed to the positive effect.

Nucleotide supplementation also has been reported to elicit significant immune effects in common carp (*Cyprinus carpio*) and channel catfish, both of which are warm water, freshwater and omnivorous species comparable to tilapia. Sakai et al. (2001) reported that nucleotides supplementation at concentrations of 0.15, 1.5, and 15 mg/fish significantly increased phagocytic activity of leukocytes. Nucleotide supplementation at 15 mg/fish also significantly increased superoxide anion production. Red-tail black shark, another fish in the family Cyprinidae, also has been reported to benefit from nucleotide supplementation. Russo and Yanong (2006) reported that the nucleotide product, Aquagen, supplemented at 2 g/kg significantly decreased *S. iniae*-related mortality in red-tailed black shark.

Unlike the previous studies with cyprinid species,Welker et al. (2011) reported that channel catfish survival after an *Edwardsiella ictaluri* challenge decreased as nucleotide supplementation increased, with a similar result in antibody titers. However, no significant differences were found in bactericidal, alternative complement, and lysozyme activities in that study. The tilapia in the present study exhibited a similar result in intracellular superoxide production, lymphocyte proliferation and lysozyme activity. Mortality in the disease challenge in this study also indicated that survival tended to decrease as the nucleotide supplementation level increased, excluding the 2.0% nucleotide supplementation level. Conversely,Welker et al. (2011) also showed that fish fed nucleotides responded with significantly higher lysozyme and bactericidal activities than fish fed the basal diet after exposure to low-water stress. Currently, the same information is not available for tilapia and may warrant further research in terms of potential effects of nucleotides in mitigating stress responses.

In comparison to studies with omnivorous fish species, there are several reports that suggest significant beneficial effects from dietary nucleotide supplementation in carnivorous species. Studies with grouper and rainbow trout (*Oncorhynchus mykiss*) have reported significant increases in plasma immunoglobulin concentrations, lysozyme activity, and superoxide anion production (Lin et al., 2009; Tahmasebi-Kohyani et al., 2011). Burrells et al. (2001a; 2001b) also found that nucleotide supplementation can increase survival in Atlantic salmon, Coho salmon (*O. kisutch*), and rainbow trout subjected to different bacterial, viral, and parasitic infections.

However, there are also several studies with carnivorous species that report varying effects of nucleotides on the immune system, similar to the results found in the present study. For example, both Cheng et al. (2011) and Li et al. (2007) reported that neutrophil oxidative reaction (NBT) of red drum did not change significantly when fed a commercial nucleotide product and/ or a purified mixture. However, Cheng et al. (2011) observed Ascogen 'P' tended to increase NBT in fish fed supplemented diets; whereas, Li et al. (2007) found both Optimûn and a purified nucleotide mixture tended to decrease NBT in fish fed supplemented diets. Li et al. (2004) also reported varying results in hybrid striped bass in which those fed Ascogen 'P' for 9 weeks showed no significant difference in mortality after *S. iniae* exposure; however, fish fed for 7 weeks experienced a significant decrease in mortality to *S. iniae*. In addition, fish fed for 6 weeks showed a significant increase in NBT while fish fed for 16 weeks showed no significant changes in NBT, lysozyme, or superoxide anion production. This suggest that time of administration may influence the effect of nucleotides on fish, especially in younger fish.

Previous research in other immunostimulants has shown that prolonged use and higher dosages do not always confer greater immune responses (Bricknell and Dalmo, 2005; Sakai, 1999). The results seen by Li et al. (2004) from feeding dietary nucleotides for different lengths of time lead to the conclusion that prolonged use of nucleotides may reduce the immune response. The results seen in the present study and Welker et al. (2011) showed lower immune responses at higher dosages also leading to the conclusion that higher dosages of nucleotide supplementation may reduce immunostimulation in certain species.

Each study, including the present one, establishes that there are some significant effects of nucleotide supplementation in various fish species. However, these can vary due to several factors such as fish species and stage of life, nucleotide components and concentration, and length of exposure. The mechanisms in which nucleotides can affect fish growth and immunity are not yet established though it is hypothesized that exogenous nucleotides may provide increased supply of nucleotides at a time when there is a high metabolic demand (Li and Gatlin, 2006; Welker et al., 2011). For example, leukocytes in particular have a high nucleotide turnover and therefore have higher nucleotide requirements than other cells (Carver and Walker, 1995; Grimble and Westwood, 2000). This may help to explain the significant benefits to the immune system often reported with nucleotide supplementation.

The present study adds to the knowledge base of research concerning nucleotide supplementation in fish. Though many of the results in growth parameters were not significant, other results suggest there is a dose-related effect in Nile tilapia. This is further demonstrated by some of the innate and adaptive immune responses. More research into understanding the specific mechanisms behind the effects of various nucleotide compounds and levels is necessary in order to develop an exact nucleotide mixture that will optimally benefit the health and growth of tilapia.

5. SUMMARY AND CONCLUSIONS

The present study evaluated various effects of supplementing graded levels of a purified dietary nucleotide mixture on growth and immune responses in tilapia. The purified nucleotide mixture used in the present study was found to significantly affect intracellular superoxide anion production and lymphocyte proliferation but not weight gain, feed efficiency or other non-specific immune responses. Therefore, the present nucleotide mixture failed to provide significant effects on growth and disease resistance of Nile tilapia but provided enhanced responses of neutrophils and lymphocytes.

REFERENCES

Agbede, S.A., Adedeji, O.B., Adeyemo, O.K., 2005. Proliferative responses of tilapia T-like lymphocytes to stimulation by concanavalin A. Afr. J. Biomed. Res. 8, 151-155.

Balfry, S.K., Shariff, M., Iwama, G.K., 1997. Strain differences in non-specific immunity of tilapia *Oreochromis niloticus* following challenge with *Vibrio parahaemolyticus*. Dis. Aquat. Organ. 30, 77-80.

Bricknell, I., Dalmo, R.A., 2005. The use of immunostimulants in fish larval aquaculture. Fish Shell. Immunol. 19, 457-472.

Burrells, C., Williams, P.D., Forno, P.F., 2001a. Dietary nucleotides: a novel supplement in fish fieeds 1. Effects on resistance to disease in salmonids. Aquaculture. 199, 159-169.

Burrells, C., Williams, P.D., Southgate, P.J., Wadsworth, S.L., 2001b. Dietary nucleotides: a novel supplement in fish feeds 2. Effects on vaccination, salt water transfer, growth rates and physiology of Atlantic salmon (*Salmo salar* L.). Aquaculture. 199, 171-184.

Carver, J.D., Walker, W.A., 1995. The role of nucleotides in human nutrition. J. Nutr. Biochem. 6, 58-72.

Cheng, Z., Buentello, A., Gatlin, D.M., 3rd, 2011. Dietary nucleotides influence immune responses and intestinal morphology of red drum *Sciaenops ocellatus*. Fish Shell. Immunol. 30, 143-147.

FAO, 2011. Cultured Aquatic Species Information Programme *Oreochromis niloticus* (Linnaeus, 1758). Available at: http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en

Fitzsimmons, K., 2000. Future trends of tilapia aquaculture in the americas. In: Costa-Pierce, B.A., Rakocy, J.E. (Eds.), Tilapia Aquaculture in the Americas. The World Aquaculture Society, Baton Rouge, LA, United States, pp. 252-264.

Fitzsimmons, K., 2006. Prospect and potential for global production. In: Lim, C.E., Webster, C.D. (Eds.), Tilapia: Biology, Culture, and Nutrition. The Hawthorne Press, Binghamton, NY, pp. 51-72.

Folch, J., Lees, M., Sloan Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-507.

Gogal, R.M., Smith, B.J., Robertson, J.L., Smith, S.A., Holladay, S.D., 1999. Tilapia (*Oreochromis niloticus*) dosed with azathioprine display immune effects similar to those seen in mammals, including apoptosis. Vet. Immunol. Immunopathol. 68, 209-227.

Grimble, G.K., Westwood, O.M.R., 2000. Nucleotides. In: Gershwin, M.E., German, J.B., Keen, C.L. (Eds.), Nutrition and Immunology: Principles and Practice. Humana Press, Inc, Totowa, NJ, pp. 135-144.

Holen, E., Bjørge, O.A., Jonsson, R., 2006. Dietary nucleotides and human immune cells. II. Modulation of PBMC growth and cytokine secretion. Nutrition. 22, 90-96.

Janossy, G., Greaves, M.F., 1971. Lymphocyte Activation 1. Response of T and B lymphocytes to phytomitogens. Clin. Exp. Immunol. 9, 483-498.

Jorgensen, J.B., Sharp, G.J.E., Secombes, C.J., Robertsen, B., 1993. Effect of a yeast-cell-wall glucan on the bactericidal activity of rainbow trout macrophages. Fish Shell. Immunol. 3, 267-277.

Lehninger, A.L., Nelson, D.L., Cox, M.M., 2005. Principles of Biochemistry, 4th ed. W.H. Freeman, New York.

Li, M.H., Lim, C.E., Webster, C.D., 2006. Chapter 14. Feed formulation and manufacture. In: Lim, C.E., Weber, G.M. (Eds.), Tilapia: Biology, Culture, and Nutrition. The Hawthorne Press, Binghamton, NY, pp. 517-546.

Li, P., Lewis, D.H., Gatlin, D.M., 3rd, 2004. Dietary oligonucleotides from yeast RNA influence immune responses and resistance of hybrid striped bass (*Morone chrysops x Morone saxatilis*) to Streptococcus iniae infection. Fish Shell. Immunol. 16, 561-569.

Li, P., Burr, G.S., Goff, J., Whiteman, K.W., Davis, K.B., Vega, R.R., Neill, W.H., Gatlin, D.M., 2005. A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). Aquacult. Res. 36, 1120-1127.

Li, P., Gatlin, D.M., 3rd, 2006. Nucleotide nutrition in fish: Current knowledge and future applications. Aquaculture. 251, 141-152.

Li, P., Gatlin, D.M., 3rd, Neill, W.H., 2007. Dietary supplementation of a purified nucleotide mixture transiently enhanced growth and feed utilization of juvenile red drum, *Sciaenops ocellatus*. J. World Aquacult. Soc. 38, 281-286.

Li, P., Wen, Q., Gatlin, D.M., 2009. Dose-dependent influences of dietary β-1,3-glucan on innate immunity and disease resistance of hybrid striped bass *Morone chrysops*×*Morone saxatilis*. Aquacult. Res. 40, 1578-1584.

Lin, Y.H., Wang, H., Shiau, S.Y., 2009. Dietary nucleotide supplementation enhances growth and immune responses of grouper, *Epinephelus malabaricus*. Aquacult. Nutr. 15, 117-122.

Mackie, A.M., Adron, J.W., 1978. Identification of inosine and inosine 5'-monophosphate as the gustatory feeding stimulants for the turbot, *Scophthalmas maximus*. Comp. Biochem. Physiol. 60A, 79-83.

Martins, M.L., Vieira, F.N., Jeronimo, G.T., Mourino, J.L., Dotta, G., Speck, G.M., Bezerra, A.J., Pedrotti, F.S., Buglione-Neto, C.C., Pereira, G., Jr., 2009.

Leukocyte response and phagocytic activity in Nile tilapia experimentally infected with *Enterococcus* sp. Fish Physiol Biochem. 35, 219-222.

Mateo, C.D., 2005. Aspects of Nucleotide Nutrition in Pigs, PhD Dissertation. South Dakota State University, Brookings.

Miller, N.W., Clem, L.W., 1988. A culture system for mitogen-induced proliferation of channel catfish (*Ictalurus punctatus*) peripheral blood lymphocytes. J. Tissue Cult. Meth. 11, 69-73.

Nematipour, G.R., Brown, M.L., Gatlin, D.M.I., 1992. Effects of dietary carbohydrate: lipid ratio on growth and body composition of hybrid striped bass. J. World Aquacult. Soc. 23, 128-132.

Pickering, L.K., Granoff, D.M., Erickson, J.R., Masor, M.L., Cordle, C.T., Schaller, J.P., Winship, T.R., Paule, C.L., Hilty, M.D., 1998. Modulation of the immune system by human milk and infant formula containing nucleotides. Pediatrics. 101, 242-249.

Pirgozliev, V., Acamovic, T., Bedford, M.R., 2009. The effect of supplemental dietary nucleotides when fed to young chickens on performance and nutrient utilisation. Brit. Poultry Abstracts. 5, 21-22.

Ramadan, A., Atef, M., Affif, N.A., 1991. Effect of the biogenic performance enhancer (Ascogen 'S') on growth rate in tilapia fish. Acta Vet. Scand. 87, S304-S306.

Ramadan, A., Affif, N.A., Moustafa, M.M., Samy, A.M., 1994. The effect of ascogen in the immune response of Tilapia fish to *Aeromonas hydrophila* vaccine. Fish Shell. Immunol. 4, 159-165.

Rudolph, F.B., 1994. Introduction. In Symposium; Dietary nucleotides: a recently demonstrated requirement for cellular development and immune function. J. Nutr. 124, 1431S-1432S.

Rumsey, G.L., Siwicki, A.K., Anderson, D.P., Bowser, P.R., 1994. Effect of soybean protein on serological response, non-specific defence mechanisms, growth, and protein utilization in rainbow trout. Vet. Immunol. Immunopathol. 41, 323-339.

Russo, R., Yanong, P.E., 2006. Dietary beta-glucans and nucleotides enhance resistance of red-tail black shark (*Epalzeorhynchos bicolor*, fam. Cyprinidae) to *Streptococcus iniae* infection. J. World Aquacult. Soc. 37, 298-306.

Sakai, M., 1999. Current research status of fish immunostimulants. Aquaculture. 172, 63-92.

Sakai, M., Taniguchi, K., Mamoto, K., Ogawa, H., Tabata, M., 2001. Immunostimulant effects of nucleotide isolated from yeast RNA on carp, *Cyprinus carpio* L. J. Fish Dis. 24, 433-438.

Saurabh, S., Sahoo, P.K., 2008. Lysozyme: an important defence molecule of fish innate immune system. Aquacult. Res. 39, 223-239.

Sealey, W.M., Gatlin, D.M., 2002. In vitro manipulations of vitamin C and vitamin E concentrations alter intracellular O−2 production of hybrid striped bass (*Morone chrysops*×*Morone saxatilis*) head-kidney cells. Fish Shell. Immunol. 12, 131-140.

Secombes, C.J., 1990. Isolation of salmonid macrophages and analysis of their killing activity. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S., van Muiswinkel, W.B. (Eds.), Techniques of Fish Immunology. SOS Publications, Fair Haven, NJ, pp. 137-154.

Shelton, W.L., Popma, T.J., 2006. Biology. In: Lim, C.E., Webster, C.D. (Eds.), Tilapia: Biology, Culture, and Nutrition. The Hawthorne Press, Binghamton, NY, pp. 1-50.

Shepard, B.S., Weber, G.M., Vijayan, M.M., Seale, A., Riley, L.G., Rodriguez, M.F., Richman, N.H.I., Hirano, T., Grau, E.G., 2006. Control of growth: development and prospects. In: Lim, C.E., Webster, C.D. (Eds.), Tilapia: Biology, Culture, and Nutrition. The Hawthorne Press, Binghamton, NY, pp. 73-138.

Siwicki, A.K., Anderson, D.P., Rumsey, G.L., 1994. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection agains furunculosis. Vet. Immunol. Immunopathol. 41, 125-139.

Solis, J.C., Santerre, A., Pérez, M.I.G., Orozco, R.R., Zaitseva, G., 2007. A comparative study of phagocytic activity and lymphoproliferative response in five varieties of tilapia *Oreochromis* spp. J. Fish Biol. 71, 1541-1545.

Tahmasebi-Kohyani, A., Keyvanshokooh, S., Nematollahi, A., Mahmoudi, N., Pasha-Zanoosi, H., 2011. Dietary administration of nucleotides to enhance growth, humoral immune responses, and disease resistance of the rainbow trout (*Oncorhynchus mykiss*) fingerlings. Fish Shell. Immunol. 30, 189-193.

Watanabe, W.O., Losorde, T.M., Fitzsimmons, K., Hanley, F., 2002. Tilapia production systems in the Americas: technological advances, trends, and challenges. Rev. Fish. Sci. 10, 465-498.

Welker, T.L., Lim, C., Yildirim-Aksoy, M., Klesius, P.H., 2011. Effects of dietary supplementation of a purified nucleotide mixture on immune function and disease and stress resistance in channel catfish, *Ictalurus punctatus*. Aquacult. Res. doi:10.1111/j.1365-2109.2010.02794.x.

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